MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD-MAPPING

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I. Introduction

During this quarter a variety of experiments were performed which extended our initial observations on the hindlimb extension and flexion torque produced by microstimulation of the lumbar spinal cord. In our initial observations it was shown that stimulation with a single microelectrode deep within the ventral horn produced extension torque of the hindlimb about the knee joint. The magnitude of these responses could be greatly increased by careful selection of location, stimulus parameters, and by the placement of additional stimulating microelectrodes in the motor pool of the hindlimb extensors. The use of multiple stimulating electrodes enhance the magnitude of the response while, also reducing the current density at each electrode tip. The addition of one or more electrodes immediately raise a number of important questions. Such as: What is the optimal number of electrodes and the optimal distance of separation? Do numbers of electrodes and distance of separation, interact with stimulus parameters and patterns of stimulation? One important consideration in addition to enhancing magnitude of the response is the reduction in response fatigue. Our focus during this quarter was to examine in detail the types of responses produced with various patterns of multi-microelectrode stimulation and the optimal distance separating electrodes which may reduce fatigue and produced enhanced responses.

II. Hindlimb Extension Torque Produced by L6 Spinal Cord Stimulation with Multi-microelectrodes.

METHODS

Since this is the final report for this particular contract the methods used in this series of studies will be summarized in detail below. These methods, with minor variations have been used throughout this contract period.

A. Animal Preparation and Experimental Setup

Seven adult male cats (3.2 kg to 4.7 kg) were studied under pentobarbital anesthesia (20 to 25 mg/kg, I.V. supplemented as necessary). Blood pressure, body temperature, and urine output were monitored throughout the experiments. Intravenous fluids (5% dextrose in saline) were administered (25 - 50 cc/hour). The spinal cord was exposed from L4 to S2 via a dorsal laminectomy. The dura mater was opened and each lumbosacral segment was identified. The spinal cord segments were determined by first identifying the seventh lumbar and the first sacral spinal nerves as they exited the vertebral column [1]. The dorsal root was then followed back to their origins at the S1 and L7 segments of the spinal cord. The remaining segments were then identified from their relative position to the L7 and S1 segments. The animal was mounted in a modified Narishige "Eccles" spinal cord frame in which the hip was supported by metal pins, and the spinal facet at the rostral end of the laminectomy secured with a clamp. The skin, cut midsagittally from L4 to S3, was tied along each margin to form a pool that was filled with warmed (35° to 37°C) mineral oil. The left tibia bone was exposed and an aluminum bar was attached parallel to the tibia by two screws. A strain gauge full-bridge rotational torque sensor (Eaton-Lebow 2120-500) was fixed to the aluminum bar with the sensor shaft aligned with the knee joint axis. The joint angle was fixed at 120° allowing both flexion and extension torques to be generated and detected. The torque sensor measured isometric torque

about the knee joint. The sensor output was plotted with flexion as positive torque and extension as negative torque. The torque signal was nulled, amplified and calibrated (Gould model 13-4615-50), and displayed on an oscilloscope. The conditioned torque signal was then captured via a National Instrument AT-MIO-16DE-10 A/D board in a PC (Dell XPS P90C) running LabVIEW 5.0 for Windows, and was also recorded on tape and displayed on a chart recorder (Gould). A train of constant current, biphasic pulses (0.2 ms pulse width, 40 Hz train for 30 s) were delivered via a stimulation isolation unit (Bak Electronics) to the spinal cord for each microelectrode. Train durations of 30 s were followed by 120 s without stimulation to allow for nervous system and end organ recovery. The computer system was triggered to collect data each time the stimulator (Grass S88) was turned on. Fifty seconds of peri-stimulus data, (including 5 sec of pretriggered data) were collected at 2000 samples/s.

B. Experimental Protocol

The left side of the L6 spinal cord segment was probed with a one dimensional rostral-caudal electrode array consisting of four fine-tipped (300 to 400 μm^2 surface area) activated-iridium microelectrodes (Microprobe Inc.) with an inter-electrode separation of 0.5 mm or 1.0 mm. The penetration of the microelectrode array was always started at the center of the dorsal root entry zone (DREZ) of L6 segment, then the microelectrodes were withdrawn and moved 200 to 400 μm medial/lateral to an adjacent location where the testing was repeated. Successive penetrations were made as long as the animal's physiological condition permitted (usually 12 to 24 hours). This microelectrode array was advanced from the dorsal surface of the spinal cord in 200 μm increments.

At each incremental stop, a train of constant-current, biphasic pulses (100 μA intensity) was delivered separately through each microelectrode to find the effective locations where measurable knee joint torque could be produced. In the locations where large extension torques were produced by stimulating a single electrode, the influences of also stimulating one to three additional electrodes in combination, and of modifying stimulus interleave time (i.e. between electrodes) on the resultant isometric extension torque produced, were also investigated. The microelectrode array tip depths tested were between 2.6 mm to 5.0 mm from the spinal cord surface. First, the results of combining electrodes using no stimulus interleave time (i.e. simultaneous activation) were tested at different stimulus intensities (from 5 μA to 100 μA) with four different electrode combinations (single electrode only, electrode pairs, three electrodes and four electrodes). The influences of stimulus interleave times (i.e. sequential activation) were then tested with two electrodes activated at from 0 ms to 12.0 ms of interleave time; and with three electrodes sequentially interleaved one by one at from 0 ms to 8.0 ms apart. During interleaving, the stimulus intensity for each electrode was set below 100 μA to produce an intermediate extension torque response less than the maximum response evoked by that electrode at 100 μA. The order of presentation of the different stimulus intensities and interleave times was randomized to minimize order influence.

C. Data Analysis

The magnitude of isometric extension torque was represented by the mean torque (T_m) generated during the first 12 s of microstimulation, since fatigue usually began to occur after 12 s to 15 s of stimulation. To show the influence of stimulus interleave time, the knee extension torque evoked by interleaved stimulation was normalized to that evoked by stimulation without interleave

in the same location. To determine how muscle fatigue was improved or worsened by interleaving a relative fatigue (R_f) index was defined.

$$R_f = (T_p - T_e)/ T_m \qquad (1)$$

where T_p is the peak extension torque produced by the 30 s stimulation train and T_e is the extension torque remaining at the end of the 30 s microstimulation.

At the end of each experiment the spinal cord was fixed with formalin, sectioned on a cryostat and the electrode positions determined histologically.

RESULTS

A. Influence of Electrode Combination

The influence of stimulating two electrodes simultaneously was either additive, facilitatory or in some instances inhibitory. Fig.1 shows that differing responses that could be produced when electrode B was paired with electrode A or D. Electrode A was 0.5 mm rostral to B and electrode D was 1.0 mm caudal. All three electrodes were at the same depth (3.8 mm) from the surface of the L6 spinal cord and penetrated the cord at the DREZ. Note that A+B reduced the response generated by A alone, but B+D enhanced the response produced by either B or D.

A typical torque response evoked by microstimulation of a combination of three or four electrodes is shown in Fig.2. The four electrodes, A, B, C and D are aligned in the rostral-caudal direction with electrode A being the most rostral. The distance between each electrode is 0.5 mm. They are all at the same depth of 4.2 mm from the surface of L6 spinal cord and penetrated the cord 300 μm medial to the DREZ. The extension torque produced by microstimulating three or four electrodes simultaneously is enhanced, compared with the response evoked by any single electrode stimulation. But the inhibitory effect (c.f. Fig. 1) can also be seen in some of the combinations (e.g., B+C+D, not shown). Note that one combination (A+B+C) produces a graded, nearly linear stimulus response relationship in contrast to the larger, but saturated response produced by A+B+C+D. Yet at 100 μA intensity, both (A+B+C and A+B+C+D) produced identical responses.

B. Influence of Stimulus Interleave Time

Fig.3 shows that the extension torque, produced by paired microelectrodes, changes with the stimulus interleave time. Note that a 40 Hz train produces a pulse every 25 ms; therefore, the maximum interleave time for a pair would be 12.5 ms and 8.33 ms for a triad (25ms/2=12.5ms, 25ms/3=8.33ms). Although the extension torques produced can vary from different experimental trials, the influences of stimulus interleave time on the torque produced were seen to be always the same for each electrode separation (filled circles for 0.5 mm separation and open circles for 3.0 mm). The extension torques produced by electrode separation 0.5 mm were always reduced after the stimulation was interleaved, and always increased if the electrode separation was 3.0 mm.

To show how electrode separation (0.5, 1.0, 2.0, 3.0 mm) and stimulus interleave time influence the knee extension torque produced by the sequential activation of paired microelectrodes, torque was normalized. Normalized torque and the relative fatigue index are shown together in Fig.4. Note that each electrode was being stimulated as 40 Hz. In all cats tested, when the electrode distance was 0.5 mm, the extension torque evoked by an interleaved stimulation of at least 1.5 ms dropped by over 50% compared with the response to simultaneous stimulation (i.e., at interleave time of 0 ms).

This initial drop became less and less as the electrode separation increased to 1 mm and then to 2.0 mm. At 3 mm, sequential delays of as little as 1.5 ms produced a torque response which exceeded that produced by simultaneous stimulation by about 50% on average. There was no significant ($P>0.05$, linear regression with 95% confidence) improvement on relative fatigue for all interleave times tested with different electrode distances.

The influences of stimulus interleave time on torques produced by a microelectrode triad (0.5 mm spacing) are shown in Fig.5. The results were similar to that produced by paired electrode stimulation (c.f., Fig.4) at the 0.5 mm electrode spacing. There was no significant ($P>0.05$, linear regression with 95% confidence) improvement on the relative fatigue index. The extension torque again dropped over 50% when compared with its response to simultaneous stimulation.

DISCUSSION AND CONCLUSION

In this study, we investigated the influences of the number of electrodes and the stimulus interleave time on the extent of isometric knee extension torque produced by multi-microelectrode stimulation of the cat L6 spinal cord. Depending on the pairing of the electrodes, the knee extension torque response evoked by multi-microelectrode stimulation could be facilitatory or inhibitory when compared with that evoked by each electrode alone. By interleaving the stimuli to two or three microelectrodes, the relative fatigue index was neither improved nor diminished. Compared with the torque response evoked by simultaneous stimulation, the extension torque evoked by sequential stimulation of microelectrode pairs decreased when electrode distance was less than 2.0 mm and increased when electrode was 3.0 mm.

The rostral-caudal distance between the electrode pairs influenced the knee extension torque evoked by the interleaved simulation. The torque response was reduced for electrode distances less than 2 mm and this reduction becomes less and less as the electrode distance increased. The activation of inhibitory interneurons might partially contribute to this reduction. However, because this reduction was produced by stimulation at different locations with different stimulus intensities and only induced by the stimulus interleaving, the interpretation of inhibitory interneuron activation is not so convincing. A more reasonable explanation for this reduction could be the spatial summation induced by simultaneous stimulus delivered from two close electrodes. Fig.6 shows the spatial summation schematically. The shadowed area in Fig.6A is the excitation region induced by each single electrode, and the area outside the shadow but within the dashed circle is the sub-threshold region. With the stimulation parameters we used, we assumed that the shadowed area had a radius less than 0.5 mm [3][4]. Spatial summation excitation can be induced in the overlapped area of the two sub-threshold regions by simultaneous stimulus delivered from the two electrodes. Fig.6 B, C and D show the potential distribution induced by simultaneous stimuli and interleaved stimuli at the time marked on the left of these figures. Because we used the interleave time from 1.5 ms to 12 ms (which is longer than refractory period), the interleaved stimuli shown in Fig.6 C and D can only excite the neurons and axons within the shadowed area. However, the simultaneous stimuli shown in Fig.6 B is able to excite not only those shadowed areas but also the overlapped area of the two dashed circles. Therefore, the extension torque response was always reduced by stimulus interleaving because of the lack of spatial summation for electrode distance less than 2.0 mm (c.f., Fig.4). This summation becomes less and less as the two electrode move apart. Based on previous studies [3][4],

an electrode separation of 3 mm is a distance that is unlikely to have a spatial summation when the stimulus intensity is below 100 μA, so the enhanced responses at 3mm separation as shown in Fig.4 could be caused by activation of excitatory interneurons. If the use of interleaved stimulation is necessary to improve muscle fatigue as suggested by other researchers [2], then spatial summation can not be used. Our data from the interleaved stimulation suggest that an electrode separation in the rostral-caudal direction larger than 2 mm might activate excitatory interneurons to compensate for the loss of spatial summation in the interleaved stimuli.

To show the improved fatigability, we did not use the difference between the initial value of a torque response and its value at the end of stimulation; rather we used a relative fatigue index. We made this choice because: (1) The extent of torque decrease at the end of stimulation is more of a concern in the design of a stimulation control strategy to produce a smooth and constant muscle contraction than attempting to maintain an initial torque value during the time of stimulation; (2) We know from our previous studies that the extent to which torque decreases at the end of stimulation will increase with an increasing torque response. So, the relative fatigue index used in this study monitored the change of the torque response during the stimulation relative to the amplitude of the torque response evoked. An improvement of the relative fatigue index indicates that a knee extension torque response was evoked more smoothly and with less overshoot by microstimulation, but this expected improvement was not seen in the data reported here.

The stimulation frequency in this study was fixed at 40 Hz based on our previous investigations that balanced the peak torque response and any resultant fatigue. We did not reduce the stimulation frequency in the interleaved case to 20 Hz, which is half of that used in simultaneous stimulation, because: (1) our previous studies show that decreasing the stimulation frequency itself will improve the relative fatigue but will reduce knee extension torque; (2) these studies should produce an improved relative fatigue index by interleaving the stimuli at 40 Hz, if the interleaving itself provides a mechanism to reduce fatigue. Although the stimuli from two different electrodes were interleaved, they were still synchronized due to the same stimulation frequency used. No improvement on the relative fatigue index was produced by such synchronized stimulation. We are now trying to use asynchronous interleaved stimulation to improve relative fatigue. The asynchronous interleaved stimulation means that two electrodes have different stimulation frequencies so that the stimulus interleave time between two electrodes changes throughout the time of stimulation. The results from asynchronous interleaved stimulation will be the topic of a future progress report.

These types of studies will continue into the next contract as will the pseudorabies tracing studies on colon and other autonomic organs.

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FIGURE CAPTIONS

Fig.1: Influence of electrode pairs on knee extension torque evoked by microstimulation of L6 spinal cord. A, B and D represent each single electrode; A+B and B+D represent the electrode combinations and penetrated the cord right at the DREZ. All electrodes are at the same depth of 3.8 mm from the surface of L6 spinal cord. The stimulation frequency is 40 Hz and pulsewidth is 0.2 ms.

Fig.2: Influence of three or four electrode combination on knee extension torque evoked by microstimulation of L6 spinal cord. A, B, C and D represent each single electrode; A+B+C and A+B+C+D represent the electrode combinations. All electrodes are at the same depth of 4.2 mm from the surface of L6 spinal cord and penetrated the cord 300 μm medial to the DREZ. The stimulation frequency is 40 Hz and pulsewidth is 0.2 ms.

Fig.3: Extension torque produced by various paired electrodes with different stimulus interleave times. Filled circles and open circles indicate electrode separations of 0.5 mm and 3.0 mm respectively. For each plot, the electrode pairs were always at the same depth from the L6 spinal cord's surface. These depths ranged from 3.6 mm to 4.8 mm. The stimulus intensity ranged from 40 μA to 80 μA.

Fig.4: Aggregate influence of stimulus interleave time and electrode separation distance on normalized isometric knee extension torque evoked by a microelectrode pair. The two electrodes were always at the same depth from the cord surface as in Fig.3. The stimulus intensity ranged from 40 μA to 80 μA. The numbers 0.5 mm, 1.0 mm, 2.0 mm and 3.0 mm represent the separation distances between the two stimulation electrodes.

Fig.5: Influence of stimulus interleave time on knee extension torque evoked by a three microelectrode combination (triad). The three electrodes were always at the same depth from the surface of L6 spinal cord. The electrode depth ranged from 4.0 mm to 4.4 mm with stimulus intensity from 25 μA to 75 μA. The number 0.5 mm represents the distance between each member of the triad.

Fig.6: Theoretical description of the spatial summation induced by simultaneous or sequential stimulation delivered from two closely positioned microelectrodes.

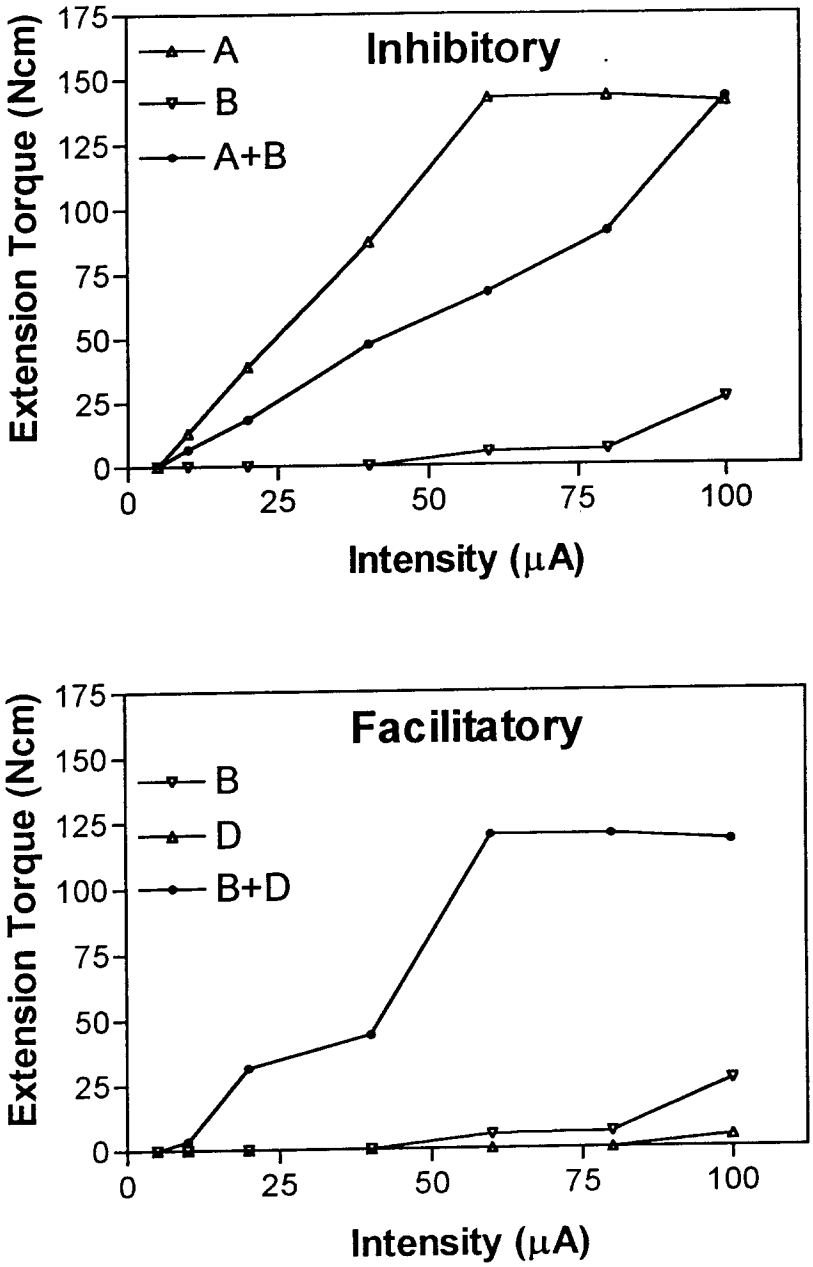
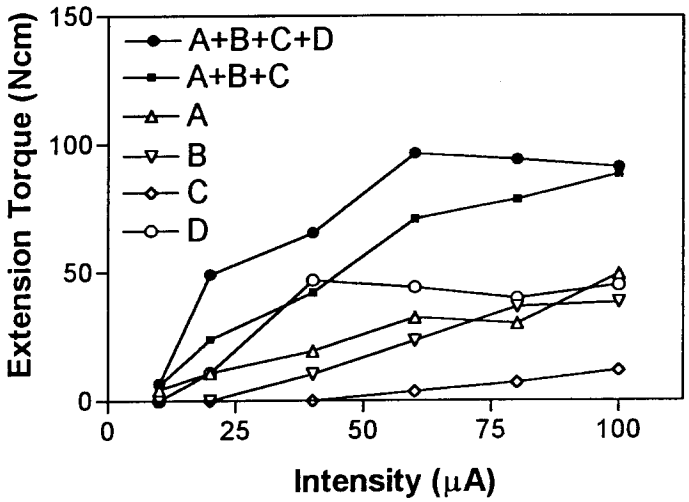


Fig.1: Influence of two electrode combination on knee extension torque evoked by microstimulation of L6 spinal cord. A, B and D represent each single electrode; A+B and B+D represent the electrode combinations. All electrodes are at the same depth of 3.8 mm from the surface of L6 spinal cord and penetrated the cord right at DREZ. The stimulation frequency is 40 Hz and pulsewidth is 0.2 ms.



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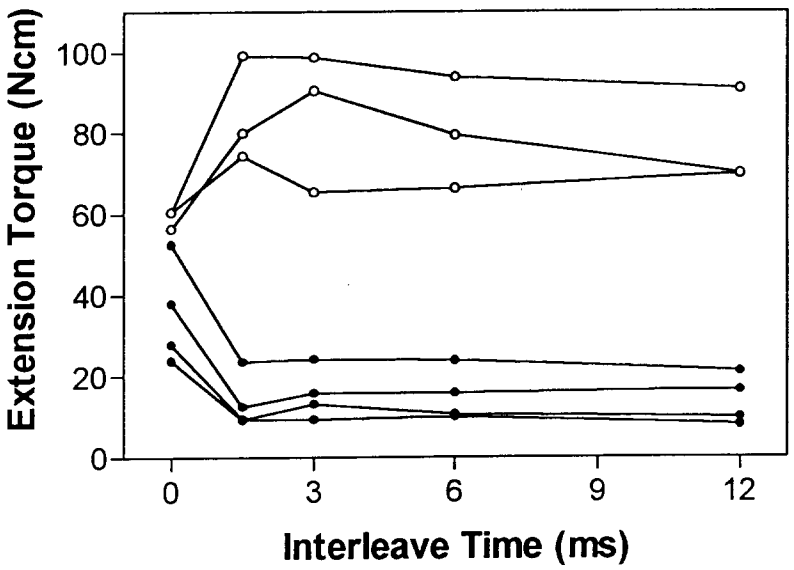

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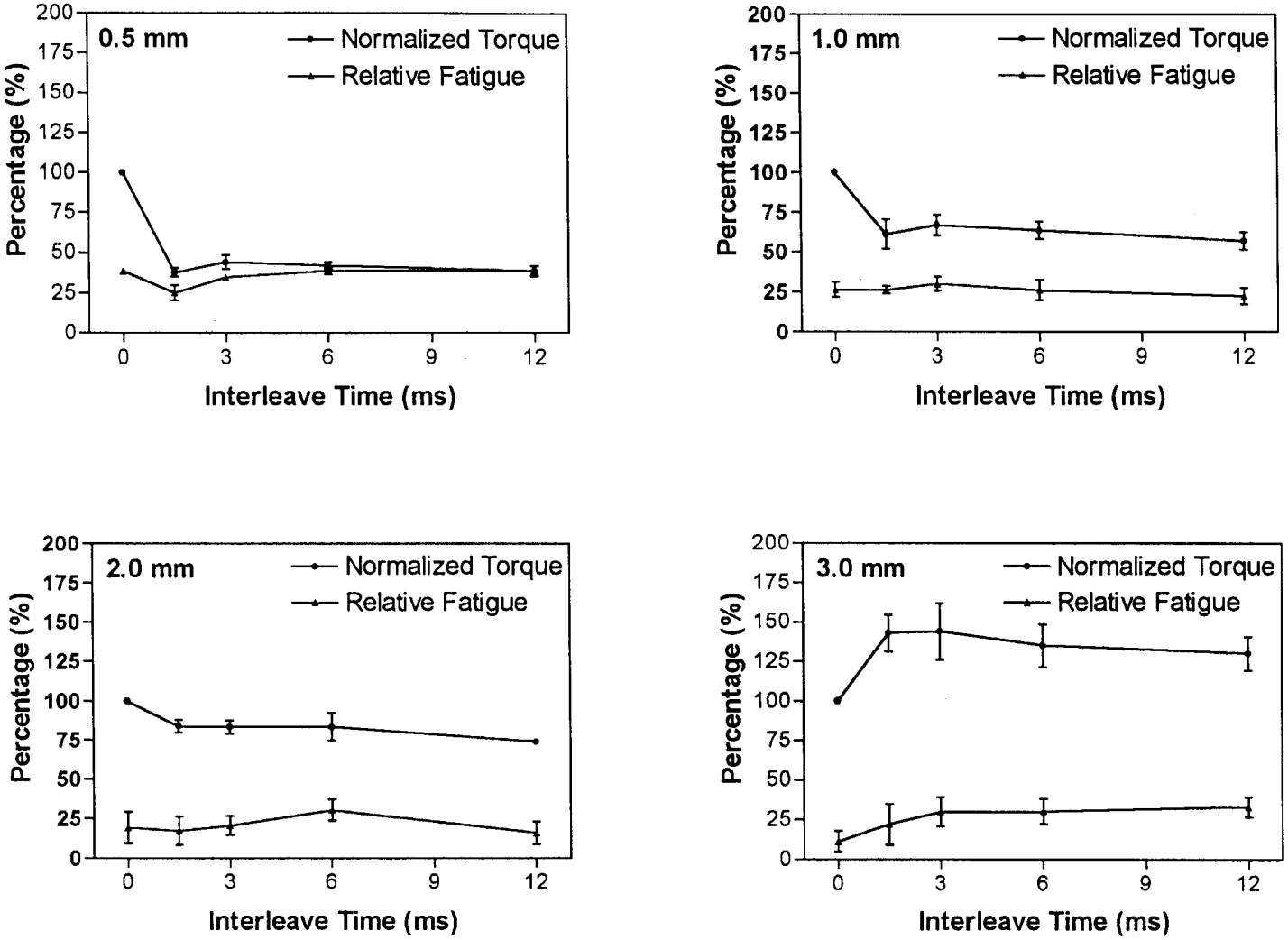

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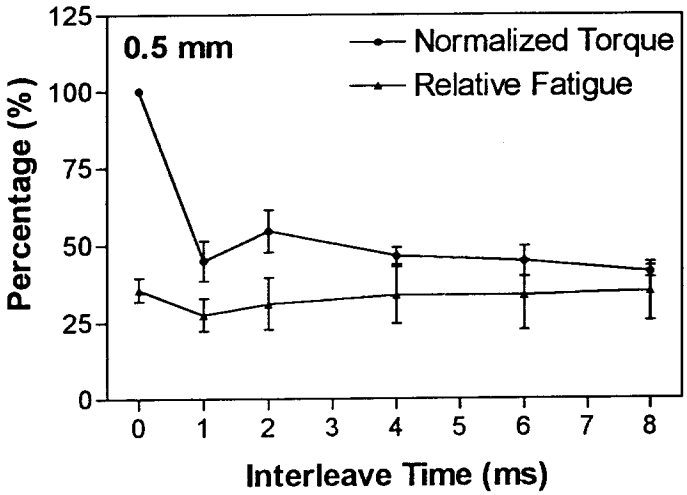

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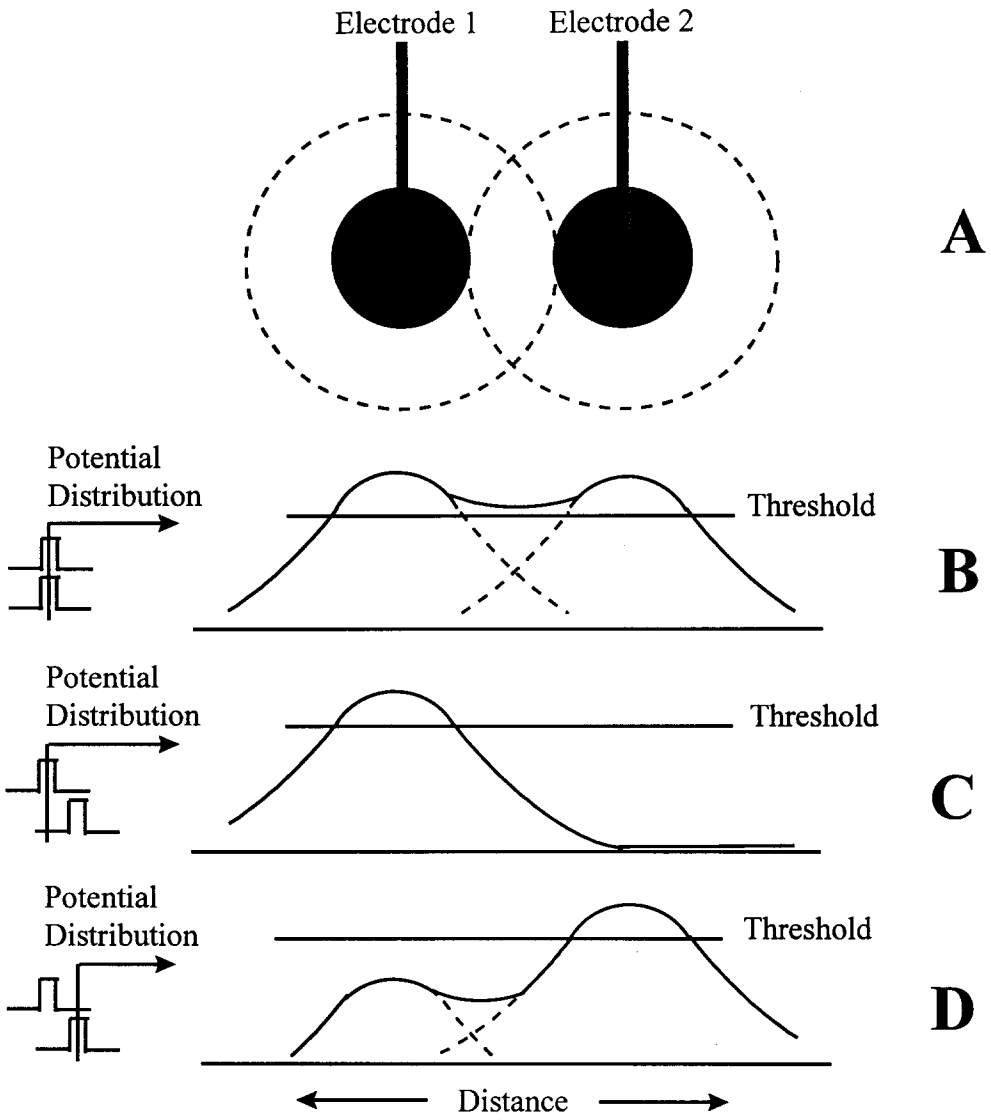

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